NO

STIC-ILL

338852

From: Goldberg, Jeanine
Sent: Tuesday, March 27, 2001 12:28 PM
To: STIC-ILL
Subject: 09/508,821

1. AMERICAN JOURNAL OF MEDICAL GENETICS, (1999 Dec 15) 88 (6) 694-9.

2. AMERICAN JOURNAL OF MEDICAL GENETICS, (7 AUG 2000) Vol. 96, No. 4, pp. P425-P425.

THANKS

Jeanine Enewold Goldberg
1655
CM1--12D11
Mailbox-- 12E12
306-5817

NC -NO EYM 3/28

American Journal of Medical Genetics (Neuropsychiatric Genetics) 96:425–428 (2000)

Letter to the Editor

Low Incidence of Myotonic Dystrophy in Chinese Hans Is Associated With a Lower Number of CTG Trinucleotide Repeats

To the Editor:

Myotonic dystrophy (DM) is an autosomal dominant neuromuscular disease characterized by myotonia, progressive weakness, and wasting of skeletal muscles, as well as a wide range of associated symptoms including cataracts, frontal balding, and intellectual impairment [Harper, 1989]. Although the biochemical mechanism underlying the disorder is still unknown, the genetic defect is believed to be the abnormal expansion of the CTG repeats in the 3′ untranslated region of the myotonin protein kinase (DMPK) gene located on chromosome 19q13.3 [Buxton et al., 1992; Harley et al., 1991, 1992]. In normal individuals, the range of the triplet repeat number falls between 5 and 35 and is variable among different populations. In patients, the repeats are usually greater than 50 and may be as high as 2,000 in severely affected cases. The number of the repeats correlates well with the age of onset and severity of the disease within families [Brook et al., 1992; Fu et al., 1992; Mahadevan et al., 1992].

Estimates of the frequency of DM in global populations vary widely, from 1 in 475 in certain regions of Canada [Bouchard et al., 1989] to 1 in 8,000 overall for Western European and North American populations [Harper, 1989]. In Japan, one in 20,000 is estimated to be affected [Osame et al.,1983], while in sub-Saharan Africa only one Nigerian affected family has been ever reported [Dada, 1973]. In China, myotonic dystrophy is scarce. Up to 1988, in total only about 100 cases were reported [Liu and Tang, 1988] and no DM patient was discovered in a general population of 126,876 people during an epidemiological survey of inheritable diseases in Sichuan Province [Zhang et al., 1990]. Also in a neurogenetico-epidemiological survey covering 152,318 people in Guangdong Province, no DM case was found [Liu et al., 1985].

Contract grant sponsor: NNSFC; Contract grant number 39993420; Contract grant sponsor: China Medical Board; Contract grant number 98-675.

*Correspondence to: Dr. Zhang Sizhong, Department of Medical Genetics, West China University of Medical Sciences, Chengdu, 610041, China. E-mail: zszg@wcums.edu.cn

Received 23 February 1999; Accepted 20 December 1999

Our previous study and a study by another group on CTG repeat polymorphism [Pan and Zhang, 1996, Tishkoff et al., 1998] found 14 and 7 alleles in 159 and 42 Chinese, respectively, and most studies on different normal populations revealed an overall trimodal distribution of the CTG repeat alleles with modes corresponding to 5, 11–17, and 19–31 CTG repeats. At the same time evidence has been accumulated that the CTG allelic distribution may explain, at least in part, the variation in frequencies of DM in different ethnic populations [Goldman et al., 1994; Krahe et al., 1995; Zerylnick et al., 1995]. To prove this, we expanded our previous study on the CTG repeat polymorphism in normal Hans who constitute 95% of the Chinese population and compared the allele distribution with the data from Caucasian, Japanese, and Southern Africa Negroid populations in connection with their incidence of myotonic dystrophy.

Three hundred unrelated Chinese Hans aged from 20 to 45 years from Chengdu, the capital city of Sichuan Province, were studied. Genomic DNA was prepared from peripheral blood cells by using a phenol-chloroform extraction procedure. PCR analysis of CTG repeat size (Fig. 1) was carried out with primers described by Guida et al. [1995]. Then the PCR products were separated on 6% non-denaturing polyacylamide gel by using DNA samples of known CTG repeat number estimated by sequencing analysis and commercial DNA markers as standards of the allele size. Statistical significance was determined by using the χ^2 test with the Yate's correction when necessary.

The variation in CTG repeats of 600 normal chromosomes is shown in Figure 2 and Table I. A total of 16 alleles ranging in size from 5 to 29 repeats are found. The most common allele has 5 repeats (35.67% of chromosomes) and the majority of alleles are in the range of 11 to 13 repeats (51.50%) with 12 repeats being more frequent (18.67%). Only two chromosomes are observed with 6 and 8 repeats, respectively, and very few alleles are found with more than 14 repeats (5.0%). It is significant that only 6 alleles (1.0%) are found with 19 repeats or more.

Comparisons of allele frequencies from present study with those in European, Japanese [Davies et al., 1992] and Southern African Negroid [Goldman et al., 1994] populations has shown (Fig. 2, Table I) , beside varia-

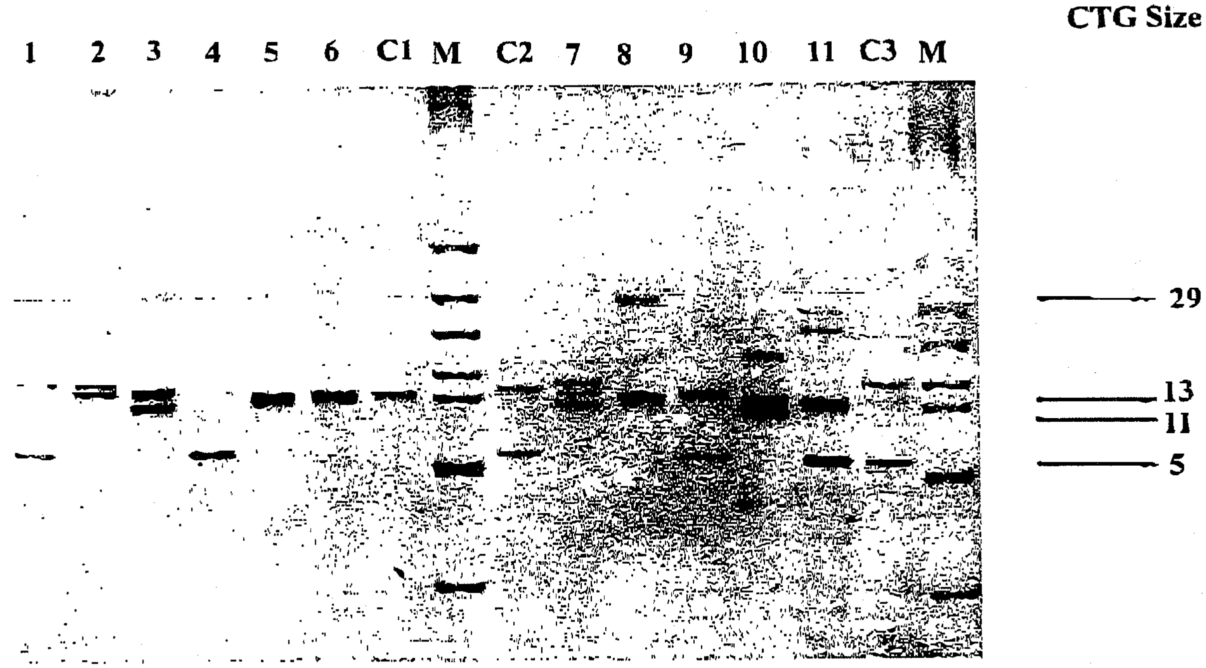

Fig. 1. DMPK CTG-repeat size in Chinese Han population. PCR amplification was carried out as described and products were resolved on a 6% polyacrylamide gel. Alleles were sized by coelectrophoresis of known size PCR products and pBR322/HaeIII marker. Numbers indicate individual Han samples. M, DNA marker; Cs, known size PCR products.

tions in frequencies of some alleles among populations, the different frequencies of alleles with larger number CTG repeats. When using 19 repeats as the cut off point, the frequencies of alleles with $(CTG)_{\geq 19}$ are 10%, 8.5%, 0.7%, and 1.0 % in Caucasian, Japanese, Southern African Negroid, and Chinese Han, respectively. The difference is highly significant between Chinese and Caucasian, Chinese and Japanese ($\chi^2 = 21.7$, P

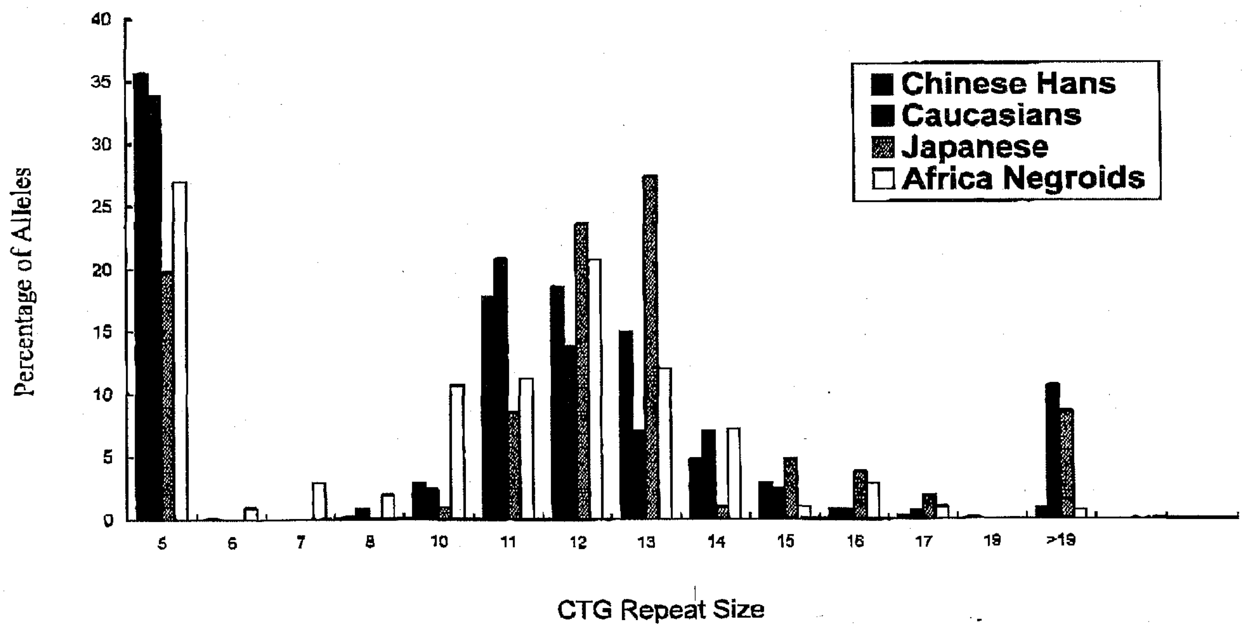

Fig. 2. Distribution of DMPK CTG repeat alleles in Chinese Han samples (black bars), Caucasians (latticed bars), Japanese (slashed bars), and Southern African Negroid (blank bars) populations.

TABLE I. Distribution of CTG Repeat Alleles in Chinese Han (C), European (E), Japanese (J), and Southern African Negroid (N) Populations With χ^2 Test and Yates' Correction When Necessary

Number of CTG repeats	Number of chromosomes				P value		
	European[a]	Japanese[a]	Negroid[b]	Chinese	CvE	CvJ	CvN
5	44	21	112	214	0.77	0.02	0.003
10	3	1	45	17	0.78	0.33	0.00001
11	27	9	47	107	0.45	0.02	0.004
12	18	25	87	112	0.21	0.28	0.42
13	9	29	50	90	0.01	0.002	0.17
14	9	1	33	28	0.37	0.11	0.0047
15	4	5	4	17	0.89	0.47	0.063
16	1	4	12	5	0.64	0.043	0.025
17	1	2	3	2	0.96	0.21	0.69
≥19	13	9	3	6	0.000	0.000	0.888
Total	130	106	420	600			

[a]Davies et al. [1992].
[b]Goldman et al. [1994].

$<10^{-4}$ and $\chi2 = 14.7$, $P<10^{-3}$, respectively). Thus, the Chinese Han is significantly different in this respect from the allele distribution of Caucasian and Japanese populations. Meanwhile it is very similar to that of Southern African Negroid where a paucity of larger repeats is also observed. No allele longer than 35 CTG repeats has been found in any of the four groups.

The present study strongly suggests that the larger CTG number, although still within the normal range, may have a greater chance of progressing into the premutation and mutation range of repeats and hence to cause DM in the next generation. The findings of Imbert et al., [1993] also suggest that the CTG repeat is the main driving force for DM gene mutation and alleles with greater than 19 CTG repeats are more unstable and thus may be the predisposing factor for DM mutations. Other population studies also provide evidence for the association between disease prevalence and CTG copy number [Tishkoff et al., 1998]. In European populations, where the frequency of alleles with $(CTG)_{\geq 19}$ is 10 %, the frequency of DM is 1/8,000, while in Southern African Negroid, for the frequency of alleles with $(CTG)_{\geq 19}$ is 0.7%, only one family of DM has been so far reported.

In China, myotonic dystrophy is a very rare disease and, from present studies, the alleles with CTG repeats greater than 19 are only 1%. This may explain, at least partially, the low incidence of DM in Chinese Hans and provides further evidence for the association of disease incidence with the frequency of alleles with higher (CTG) repeats in populations.

REFERENCES

Bouchard G, Roy R, Declos M, et al. 1989. Origin and diffusion of the myotonic dystrophy gene in the Saguenay region. Can J Neur Sci 116: 119–122.

Brook JD, McCurrach ME, Harley HG, et al. 1992. Molecular basis of myotonic dystrophy: expansion of trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. Cell 68; 799–808.

Buxton J, Shelbourne P, Davies J, et al. 1992. Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. Nature 355:547–548.

Dada TO. 1973. Dystrophia myotonica in Nigerian family. East Afr Med J 50:213–228.

Davies J, Yamagata H, Shelbourne P, et al. 1992. Comparison of the myotonic dystrophy associated CTG repeat in European and Japanese population. J Med Genet 29:766–769.

Fu YH, Pizzuti A, Fenwick RG Jr, King J, et al.1992. An unstable triplet repeat in a gene related to myotonic dystrophy. Science 255; 1256–1258.

Goldman A, Ramsay M, Jenkins T, et al. 1994. Absence of myotonic dystrophy in Southern African Negroids is associated with a significantly lower number of CTG trinucleotide repeat. J Med Genet 31: 37–40.

Guida M, Marger RS, Papp AC, et al. 1995. A molecular protocol for diagnosing myotonic dystrophy. Clin Chem 41:69–72.

Harley HG, Walsh KV, Rundle S, et al. 1991. Localization of the myotonic dystrophy locus to 19q13.2-19q13.3 and its relationship to twelve polymorphic loci on 19q. Hum Genet 87:73–80.

Harley HG, Brook JD, Rundle SA, et al. 1992. Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. Nature 355:545–546.

Harper PS.1989. Myotonic dystrophy. 2nd edition. London: WB Saunders.

Imbert G, Kretz C, Johnson K, et al. 1993. Origin of the expansion mutation in myotonic dystrophy. Nature Genet 4:72–75.

Krahe R, Eckhort M, Ogunniyi AO, et al. 1995. De novo myotonic dystrophy mutation in a Nigerian kindred. Am J Hum Genet 56:1067–1074.

Liu ZL, Tang XL, Pan XB. 1985. Epidemiological survey of inheritable neurological diseases in 152,318 individuals in Guangdong Province, China. Chinese J Neurol Mental Dis 11:337–340.

Liu ZL, Tang XL. 1988. Genetics in neurology. Beijing: People's Health Press, p 137–141.

Mahadevan MS, Tsilfidis C, Sabourin L, et al. 1992. Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. Science 255:1253–1255.

Osame M, Furusho T. 1983. Genetic epidemiology of myotonic dystrophy in Kagoshima and Okinawa districts in Japan. Rinsho Shinkeigaku 23: 1067–1071.

Pan AG, Zhang SZ. 1996. Polymorphism of CTG trinucleotide repeats of myotonic dystrophy gene in Chinese population. Chin J Med Genet 13:138–142.

Tishkoff SA, Goldman A, Calafell F, et al. 1998. A global haplotype analysis of the myotonic dystrophy locus: implication for the evolution of modern humans and for the origin of myotonic dystrophy. Mutations 62; 1389–1402.

Zerylnic C, Torroni A, Sherman SL, et al. 1995. Normal variation at the myotonic dystrophy locus in global human population. Am J Hum Genet 56:123–130.

Zhang SL, Tang XD, Zhang SZ. 1990. Epidemiological surveys of genetic diseases in general population of Sichuan, China. Chengdu: Chengdu Science and Technology University Press.

Zhang Sizhong*
Wu Hui
Pan Agen
Xiao Cuiying
Zhang Ge

Department of Medical Genetics
West China University of Medical Sciences
Chengdu, China

Hou Yiping

Institute of Forensic Medicine
West China University of Medical Sciences
Chengdu, China

Chu Jiayou

Institute of Medical Biology
Chinese Academy of Medical Sciences
Kunming, China